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Short Communications

Microimplantation of [^{3}H]proline on a single bead of ion-exchange resin

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An advantage of the use of radioautography of axonally transported proteins to trace neuroanatomical pathways is its precision and selectivity. Only cell bodies at the site of injection incorporate the precursor, so that axons of passage are not affected[1,9,11]. The selectivity of the method is thus limited only by the size of the injection site. In studies of the goldfish visual system, injection is made into the vitreous humor[10], and the use of [^{3}H]proline has been demonstrated to be a particularly suitable precursor[3,11]. The ganglion cell bodies synthesize labeled protein, which is then transported to the brain via the optic nerve.

Injection of precursor into an intracerebral site is less simple, since it is necessary to disrupt the brain substance with the shaft of a pipet or needle. If the isotope is injected hydrostatically, there may be considerable diffusion at the injection site, depending upon the amount of prior physical trauma, rate of injection and on the volume injected. To minimize these problems, we examined the feasibility of concentrating the precursor on the surface of a cation ion-exchange resin bead which was implanted by first fixing it to the tip of a fine wire. An immediate application of the method was to map the projections of the olfactory bulb in the teleost fish, *Macropodus opercularis*. The 0.5 mm wide bulbs protrude from beneath the rostral edge of the telencephalon and are readily visualized by cutting a 2 mm hole in the dorsal midline of the cranium[2]. In the experiment described below, a resin bead containing tritiated proline was implanted in the center of the visible portion of the bulb without touching the telencephalon.

Preparation of precursor-labeled beads. Ten DOWEX 50W-X8, H^+ form (200–400 mesh, Bio-Rad) beads, 60–80 μm in diameter, were set out in the center well of a glass depression slide. They had been selected for size under the dissecting microscope after having been air-dried for 5 min. The beads were washed twice with 100 μl of distilled, deionized water and then dried. Twenty μl of water containing 15–20 μCi of L[^{3}H-2,3]proline were added and the beads were dried under a flow of air. An additional 20 μl of water was added prior to final drying.

Single beads were mounted with a film of Ficoll (Pharmacia) on the flattened tip

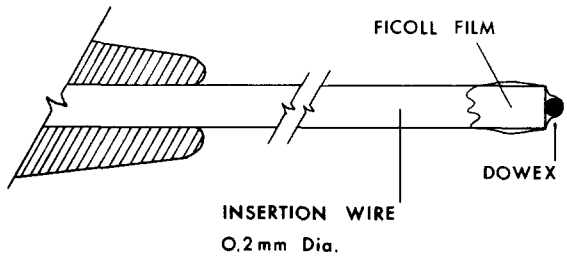

Fig. 1. Diagram of coated resin bead on the 2 cm long insertion wire, attached to a glass tubing handle.

of a 200 μm diameter insect pin (Emil Arlt, Austria; Fig. 1). The dried Ficoll film is a good adhesive which is easily loosened in water. Moreover, it is non-ionic and presumably results in less osmotic disturbance per unit weight at the implantation site than sucrose, by virtue of its higher molecular weight. The tip of the wire was dipped in a 20–30% aqueous suspension (w/v) of Ficoll, then immediately touched to the bead. The bead-wire assembly was dried briefly, then immersed momentarily in 10% Ficoll and air-dried again preparatory to implantation. The final coat of Ficoll was added to increase the strength of attachment of the bead and to decrease smearing of the isotope on superficial tissue during insertion.

The amount of radioactivity bound to the beads and amount lost in the 10% Ficoll dipping solution was determined for 3 beads, measured in the dissecting microscope to have diameters of approximately 60, 70 and 80 μm (Table I). To evaluate the radioactive content of the beads, they were dislodged from their wires by brief immersion in 1 ml of water containing two drops of the organic base, ethanolamine, in a counting vial. Immersion of the bead directly into the counting vial without the prior aqueous ethanolamine treatment resulted in falsely low counts. The beads bound about 0.25–0.5 μCi each. Loss to the remaining Ficoll solution was less than 0.1% of the total radioactivity on the beads, suggesting that the isotope had indeed been ionically bound to the resin surface. Similar amounts of radioactivity per bead were obtained in experiments in which the isotope was bound as described, but counted without the intervening Ficoll steps. The appeal of the ion-exchange bead is that a small volume will store a large amount of precursor. In this instance, the amino group of the proline is bound to the sulfonate of the resin, and is released as tissue cations displace the precursor in situ.

TABLE I

The amount of tritium label bound to individual beads of Dowex resin and the amount lost in the final Ficoll dipping solution

Bead diameter (μm)	*Disint./min*	
	Bead	*Ficoll*
80	1.75×10^6	285
70	1.54×10^6	2209
60	1.00×10^6	333

The capacity of the beads is considerably greater than was utilized in the present experiment. To establish the theoretical value, the density of the air-dried beads was measured and found to be 1.25. A 60 μm diameter bead has a mass of $4/3 \times \pi \times 30$ cu.μm $\times$ 1.25 $\times$ 10^{-9} mg/cu.μm = 0.14 μg. The stated capacity of the dry resin is 5.1 mEq./g, hence the 60 μm beads could each bind 0.72 nmole of proline. The isotope used had a specific activity of 35 μCi/nmole, so that ten 60 μm beads should have been able to bind about 250 μCi. Although the beads took up at least 25% of the added

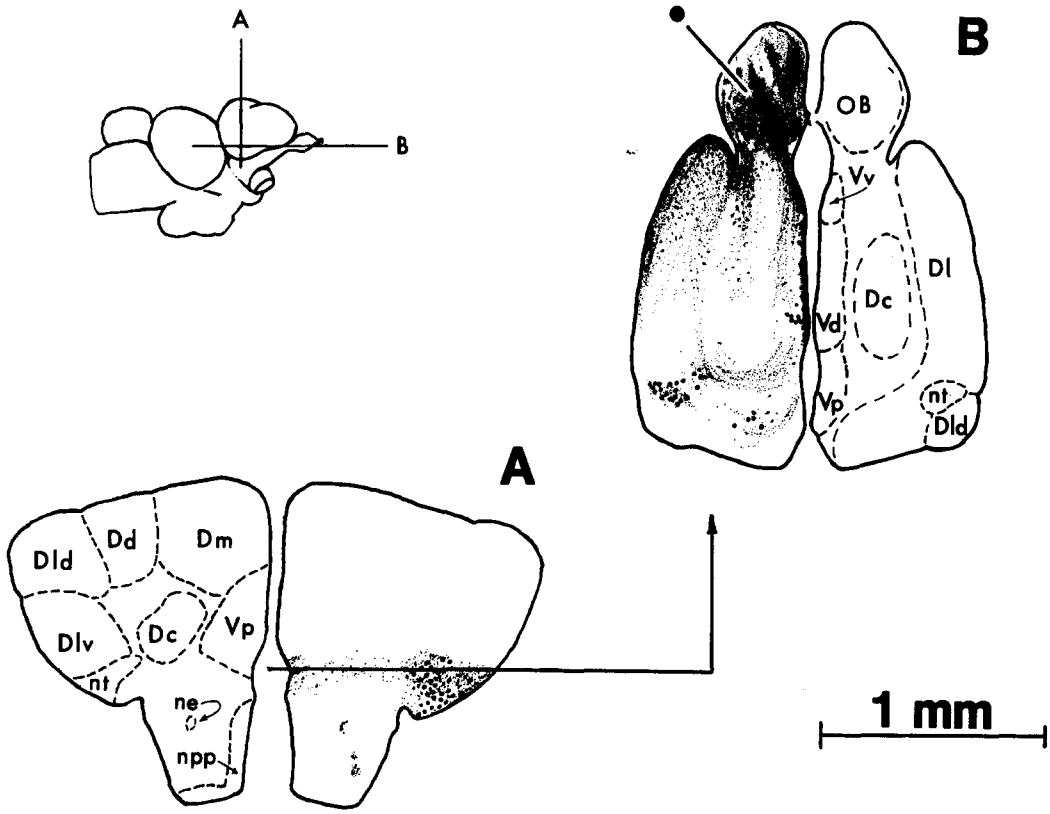

Fig. 2. Drawings of *Macropodus* brain following implantation in the left olfactory bulb of a 60 μm Dowex resin bead (solid dot), which was treated with [^{3}H]proline. Upper left: side view of the brain, 7 $\times$ life, showing the planes of sections A and B. Section A (brain R559) was caudal to the anterior commissure and rostral to the zone of coalescence of the pallium[2]; section B (brain R647) was ventral to the pallial coalescence. The distribution of silver grains is shown for the left side of the brain. Most of the label was distributed over the cells and neuropil of the left olfactory bulb and rostral telencephalon (section B). Caudally, the amount of label decreased. Sweeping patterns of diffusely labeled neuropil, suggesting fiber pathways, were interspersed with clusters of varying numbers of intensely labeled cells. The large cluster, which includes cells of nucleus taenia, is a possible principal terminus of the secondary olfactory fibers. Abbreviations: Dc, area dorsalis telencephali pars centralis; Dd, area dorsalis telencephali pars dorsalis; Dl, area dorsalis telencephali pars lateralis; Dld, area dorsalis telencephali pars lateralis dorsalis; Dlv, area dorsalis telencephali pars lateralis ventralis; Dm, area dorsalis telencephali pars medialis; Ob, olfactory bulb; ne, nucleus entopeduncularis; npp, nucleus preopticus periventricularis; Vd, area ventralis telencephali dorsalis; Vp, area ventralis telencephali pars postcommissuralis; Vv, area ventralis telencephali pars ventralis[12,13].

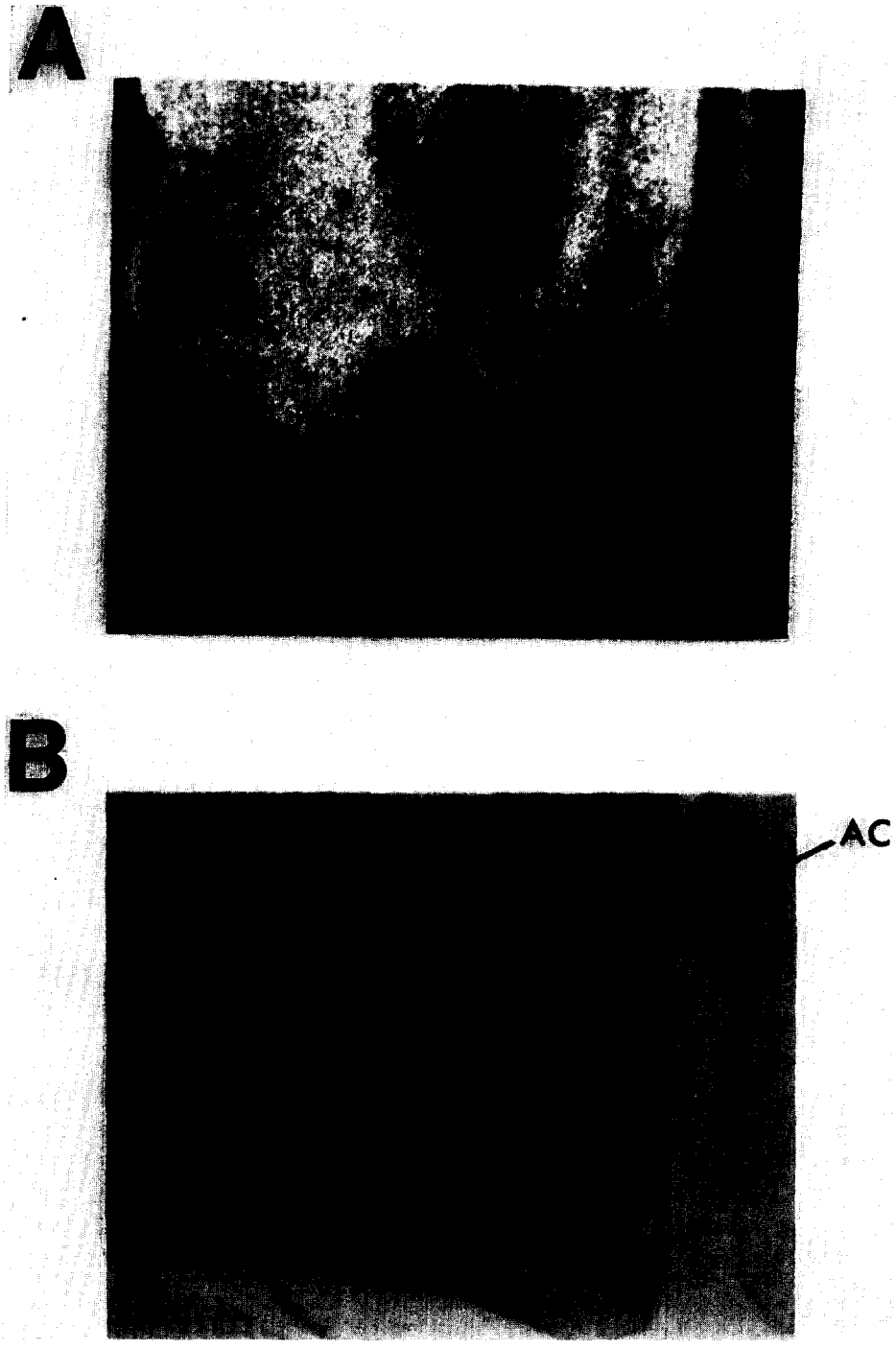

Fig. 3. A: dark-field photomicrograph showing the distribution of silver grains in the caudal portion of the left telencephalon, taken from the same section as illustrated in Fig. 2B. The dense aggregations of grains over cell bodies in the bright-field photomicrograph (B) more clearly reveal the location of the most intensely labeled cells. This section was approximately 50 μm ventral to the plane of Fig. 2A, and reveals the top of the anterior commissure (AC).

radioactivity, this amounts to only 2% of their theoretical capacity and may reflect the finite equilibrium constant of binding of the resin and the presence of small amounts of non-radioactive cations in the isotope preparation, in the water, or on the glass slide. The use of ultrapure materials may increase considerably the amount of labeled material that can be held on a single bead.

Implantation. The insertion wire was attached to a handle for freehand implantation of the bead using a microscope, or by means of a previously aligned electrode holder of a stereotaxic device. The wire tip was pushed into the olfactory bulb, held there for a few seconds to permit the Ficoll to dissolve and was then withdrawn, leaving the bead behind.

Radioautography. Adult male *Macropodus* that had received olfactory bulb implants were kept for 5 h in individual tanks prior to being sacrificed in ice-water. The brain was removed in the cranium and fixed in alcohol–formalin–acetic acid for 2 days. The tissue was then decalcified for 4 h, dehydrated, passed through several changes of cedar wood oil, and then embedded in Paraplast. The cranium was cut transversely in 8 μm sections, and prepared for radioautography[7,8]. The slides were dipped in Kodak NTB-2 emulsion, dried, and stored in darkness at 4 °C for 8 days; the emulsion was developed in Kodak Dektol for 3 min at 16 °C, washed and fixed. The sections were stained with cresyl violet or hematoxylin–eosin.

The labeling was most intense in the implanted olfactory bulb and the adjacent portion of the ipsilateral telencephalon (Figs. 2 and 3). The contralateral bulb and telencephalon showed much less intense radioactivity, and the silver grains were chiefly near the medial surface of those structures. The possibility that diffusion from the implantation site was responsible for the contralateral labeling needs to be investigated, since efferent projections to the contralateral bulb were found in the teleost *Ictalurus* by Finger[4].

In the remainder of the telencephalon, labeling occurred mainly in the medial and ventrolateral portions of the subpallium, and the basal portion of the posterior and lateral pallium (Fig. 2). Extensive areas of the dorsal, central and posterior telencephalon showed few or no silver grains. Grains occurred in dense aggregations over cell bodies. This could represent presynaptic terminals of primary afferent fibers or secondary labeling due to breakdown of transported protein and reutilization of [^{3}H] proline[5]. Grains occurring over neuropil were more evenly scattered. The intensely labeled cells in the nucleus taenia and nearby dorsalis telencephali pars lateralis[12,13] correspond topographically to areas which receive olfactory bulb projections in *Ictalurus*[4], *Cyprinus*[6], and *Gymnothorax*[14]. The results of the continuing investigation in *Macropodus* are to be described elsewhere.

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1 Cowan, W. M. and Cuénod, M., *The Use of Axonal Transportation for Studies of Neuronal Connectivity*, Elsevier, Amsterdam, 1976, 400 pp.

2 Davis, R. E., Kassel, J. and Schwagmeyer, P., Telencephalic lesions and behavior in the teleost *Macropodus opercularis*: reproduction, startle reaction, and operant behavior in the male, *Behav. Biol.*, 18 (1976) in press.
3 Elam, J. S., and Agranoff, B. W., Rapid transport of protein in the optic system of the goldfish, *J. Neurochem.*, 18 (1971) 375–387.
4 Finger, T. E., The distribution of olfactory tracts in the bullhead catfish, *Ictalurus nebulosus*, *J. comp. Neurol.*, 161 (1975) 125–142.
5 Heacock, A. M. and Agranoff, B. W., Reutilization of precursor following axonal transport of [^{3}H]proline-labeled protein.
6 Ito, H., Normal and experimental studies on synaptic patterns in the carp telencephalon, with special reference to the secondary olfactory termination, *J. Hirnforsch.*, 14 (1973) 237–245.
7 Kopriwa, B. M. and Leblond, C. P., Improvements in the coating technique of radioautography, *J. Histochem. Cytochem.*, 10 (1962) 269–284.
8 Landreth, G. E. and Agranoff, B. W., Explant culture of adult retina: effect of prior optic nerve crush, *Brain Research*, 118 (1976) 299–303.
9 Lasek, R., Joseph, B. W. and Whitlock, D. G., Evaluation of a radioautographic neuroanatomical tracing method, *Brain Research*, 8 (1968) 494–507.
10 McEwen, B. S. and Grafstein, B., Fast and slow components in axonal transport of protein, *J. Cell Biol.*, 38 (1968) 494–507.
11 Neale, J., Neale, E. A. and Agranoff, B. W., Radioautography of the optic tectum of the goldfish after intraocular injection of ^{3}H-proline, *Science*, 176 (1972) 407–410.
12 Nieuwenhuys, R., The interpretation of the cell masses in the teleostean forebrain. In R. Hassler and H. Stephan (Eds.), *Evolution of the Forebrain*, Thieme, Stuttgart, 1966, pp. 32–39.
13 Peter, R. and Gill, V., Stereotaxic atlas and technique for forebrain nuclei of goldfish, *Carrassius auratus*, *J. comp. Neurol.*, 159 (1975) 69–101.
14 Scalia, F. and Ebbesson, S. O. E., The central projections of the olfactory bulb in a teleost (*Gymnothorax funebris*), *Brain Behav. Evol.*, 4 (1971) 376–399.
15 Schwagmeyer, P., Davis, R. E. and Kassel, J., Telencephalic lesions and behaviour in the teleost *Macropodus opercularis* (L): effects of telencephalon and olfactory bulb ablation on spawning and foamnest building, *Anim. Behav.*, (1977) in press.